

WHAT IS CLAIMED IS:

1. A method for determining the amount of an analyte in a fluid sample in the presence of an interfering substance, comprising:

5 (a) providing a solid surface dual-coated with a first antibody recognizing said free analyte and a second antibody recognizing said interfering substance when bound to said analyte;

(b) contacting said dual-coated surface with said fluid sample, whereby said first antibody binds said free analyte and said second antibody binds said interfering substance bound to said analyte; and

10 (c) determining the total amount of said free analyte and said analyte bound to said interfering substance.

2. The method of claim 1, wherein the total amount of said free analyte and said analyte bound to said interfering substance in step (c) is determined by contacting the product of step (b) with a detectably labeled secondary antibody recognizing said analyte.

3. The method of claim 2, wherein the total amount of said free analyte and said analyte bound to said interfering substance is compared with a standard curve generated by using various concentrations of said purified analyte in place of said fluid sample in step (b).

4. The method of claim 1, wherein said first and second antibodies are polyclonal antibodies.

5. The method of claim 1, wherein said first and second antibodies are monoclonal antibodies.

6. The method of claim 2, wherein said secondary antibody recognizes an epitope on said analyte different from that recognized by said first antibody coated on said surface as well as different from that recognized by said interfering substance.

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7. The method of claim 2, wherein said detectable label is an enzyme conjugated to said secondary antibody.

5 8. The method of claim 7, wherein said enzyme is peroxidase.

9. The method of claim 8, wherein said peroxidase is horseradish peroxidase.

10 10. The method of claim 7, wherein said enzyme is alkaline phosphatase.

11. The method of claim 7, wherein said enzyme is detected using a substrate for said enzyme.

15 12. The method of claim 11, wherein said substrate is ortho-phenylene diamine (OPD) for the detection of said peroxidase.

13. The method of claim 1, wherein said solid surface is in a configuration of a test tube, a well, a bead, a rod, or a strip.

20 14. The method of claim 13, wherein said configured solid surface is glass, plastic, or paper.

25 15. The method of claim 14, wherein said plastic is polystyrene or polyacrylate.

16. The method of claim 1, wherein said fluid sample comprises a biological fluid.

30 17. The method of claim 16, wherein said biological fluid comprises serum or plasma.

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18. The method of claim 1, wherein said analyte is a polypeptide.

19. The method of claim 1, wherein said analyte is a receptor, and said interfering substance is a ligand for said receptor.

20. A method for accurately determining the amount of an antibody in a fluid sample in the presence of an interfering substance capable of binding to said antibody, comprising:

(a) providing a solid surface dual-coated with a first antibody recognizing said free antibody and a second antibody recognizing said interfering substance when bound to said antibody;

(b) contacting said dual-coated surface with said fluid sample, whereby said first antibody binds said free antibody and said second antibody binds said interfering substance bound to said antibody; and

(c) determining the total amount of said free antibody and said antibody bound to said interfering substance.

21. The method of claim 20, wherein the total amount of said free antibody and said antibody bound to said interfering substance in step (c) is determined by contacting the product of step (b) with a detectably labeled secondary antibody.

22. The method of claim 20, wherein the total amount of said free antibody and said antibody bound to said interfering substance is compared with a standard curve generated by using various concentrations of said purified antibody in place of said fluid sample in step (b).

23. The method of claim 20, wherein said antibody recognizes an antigen and said interfering substance is a polypeptide derived from said antigen.

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24. The method of claim 23, wherein said antigen is a receptor and said interfering polypeptide is a fragment derived from said receptor.

25. The method of claim 21, wherein said secondary antibody is raised against an immunoglobulin derived from the same species as said analyte antibody.

26. The method of claim 20, wherein said first and second antibodies are polyclonal antibodies.

27. The method of claim 20, wherein said first and second antibodies are monoclonal antibodies.

28. The method of claim 23, wherein said secondary antibody recognizes an epitope on said analyte antibody different from that recognized by said first antibody coated on said surface as well as different from that recognized by said interfering substance.

29. The method of claim 29, wherein said detectable label is an enzyme conjugated to said secondary antibody.

30. The method of claim 29, wherein said enzyme is peroxidase.

31. The method of claim 30, wherein said peroxidase is horseradish peroxidase.

32. The method of claim 29, wherein said enzyme is alkaline phosphatase.

33. The method of claim 29, wherein said enzyme is detected using a substrate for said enzyme.

34. The method of claim 33, wherein said substrate is ortho-phenylene diamine (OPD) for the detection of said peroxidase.

5 35. The method of claim 20, wherein said antibody is an anti-HER2 antibody, and said interfering substance is an extracellular domain (ECD) of HER2 oncoprotein.

10 36. The method of claim 35, wherein said anti-HER2 antibody is a murine monoclonal antibody.

37. The method of claim 36, wherein said murine anti-HER2 monoclonal antibody is 4D5.

15 38. The method of claim 37, wherein said anti-HER2 murine monoclonal antibody is a humanized version of recombinant 4D5 antibody.

39. The method of claim 38, wherein said humanized version of recombinant 4D5 anti-HER2 antibody is HERCEPTIN®.

20 40. The method of claim 39, wherein said first antibody recognizes anti-HER2, and said second antibody recognizes HER2 ECD at an epitope different from that recognized by said anti-HER2 antibody.

25 41. The method of claim 40, wherein said first antibody recognizes HERCEPTIN® and does not significantly cross-react with human IgG, and said second antibody against ECD is a polyclonal or a monoclonal antibody.

30 42. The method of claim 41, wherein said anti-HERCEPTIN® is a monoclonal antibody or a fragment derived therefrom, and said monoclonal antibody against ECD is 7C2.

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43. The method of claim 42, wherein said anti-HERCEPTIN® monoclonal antibody is AMER5.

44. A method of accurately determining the amount of circulating anti-HER2 antibodies according to any one of the claims 35-39, wherein said fluid sample is serum or plasma derived from a cancer patient undergoing anti-HER2 therapy.

45. The method of claim 44, wherein said cancer is breast cancer overexpressing HER2.

46. A method for determining the amount of an analyte in a fluid sample in the presence of an interfering substance, comprising:

(a) providing a solid surface dual-coated with a first capture reagent recognizing said free analyte and a second capture reagent recognizing said interfering substance when bound to said analyte;

(b) contacting said dual-coated surface with said fluid sample, whereby said first capture reagent binds said free analyte and said second capture reagent binds said interfering substance bound to said analyte; and

(c) determining the total amount of said free analyte and said analyte bound to said interfering substance.

47. A method for determining the amount of an analyte in a fluid sample in the presence of an interfering substance, comprising:

(a) providing a first antibody recognizing said free analyte and a second antibody recognizing said interfering substance when bound to said analyte;

(b) contacting said first antibody and said second antibody with said fluid sample, whereby said first antibody binds said free analyte and said second antibody binds said interfering substance bound to said analyte; and

(c) determining the total amount of said free analyte and said analyte bound to said interfering substance.

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48. The method of claim 47 wherein the total amount of said free analyte and said analyte bound to said interfering substance in step (c) is determined by fluorescence resonance energy transfer (FRET).

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49. A kit for accurately determining the amount of an anti-HER2 antibody in serum or plasma in the presence of HER2 ECD, comprising:

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(a) a solid surface coated with a first antibody recognizing said free anti-HER2 antibody and a second antibody recognizing said HER2 ECD when bound to said anti-HER2 antibody; and

(b) a detectably labeled secondary antibody recognizing said anti-HER2 antibody at an epitope different from that recognized by said first antibody as well as different from that recognized by said HER2 ECD.

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50. The kit of claim 49, wherein said solid surface is in a configuration of a test tube, a well, a bead, a rod, or a strip.

51. The kit of claim 50, wherein said configured solid surface is glass, plastic, or paper.

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52. The kit of claim 51, wherein said plastic is polystyrene or polyacrylate.

53. The kit of claim 49, wherein said anti-HER2 antibody is a murine monoclonal antibody.

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54. The kit of claim 53, wherein said murine anti-HER2 monoclonal antibody is 4D5.

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55. The kit of claim 54, wherein said anti-HER2 murine monoclonal antibody is a humanized version of recombinant 4D5 antibody.

56. The kit of claim 55, wherein said humanized version of recombinant 4D5 anti-HER2 antibody is HERCEPTIN®.

57. The kit of claim 49, wherein said first antibody recognizes HERCEPTIN® and does not significantly cross-react with human IgG, and said second antibody against HER2 ECD is a polyclonal or a monoclonal antibody.

58. The kit of claim 57, wherein said anti-HERCEPTIN® is a monoclonal antibody or a fragment derived therefrom, and said monoclonal antibody against HER ECD is 7C2.

59. The kit of claim 58, wherein said anti-HERCEPTIN® monoclonal antibody is AMER5.

60. The kit of claim 49, wherein said serum or plasma is derived from a cancer patient undergoing anti-HER2 therapy.

61. The kit of claim 60, wherein said cancer is breast cancer overexpressing HER2.

62. A microtiter plate for accurately determining the amount of an anti-HER2 antibody in serum or plasma in the presence of HER2 ECD, comprising:

(a) a first antibody recognizing said free anti-HER2 antibody and a second antibody recognizing said HER2 ECD when bound to said anti-HER2 antibody; and

(b) wherein said first and said second antibodies are coated on the surface of said microtiter plate.